27. Use of an immobilized antimicrobial for intervention of environmental sources of microbial populations in the homes of mold-sensitive subjects and subsequent monitoring of the presentation of allergic symptoms

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INTRODUCTION

Hypersensitivity resulting from the exposure to fungi or other microbially-sourced antigens has been well documented. Temporary relief from the allergic discomforts is possible but causal prophylaxis requires the elimination of microbial reservoirs. An immobilized antimicrobial has been shown to transform surfaces into active control devices that continuously inhibit microorganisms. This sustained inhibition of microbes contacting treated surfaces effectively controls airborne microbial concentrations.

There has been mounting scientific evidence that indoor air pollution is more serious than outdoor pollution and has greater significance for the health of individuals. This is magnified by the fact that many people spend over 90% of their time indoors [16]. People exposed to indoor air pollutants for the longest period of time such as the young, elderly, or chronically ill, may be most susceptible to the adverse effects of indoor air pollutants. Most notable among these pollutants are radon, tobacco smoke, biological contaminants, carbon monoxide, nitrogen oxide, organic gases, formaldehyde, pesticides, asbestos and lead [14]. Biological contaminants, especially airborne microorganisms, are becoming increasingly significant as indoor air pollutants.

A number of surveys of mold growth in homes have been conducted over the past 35 years [3, 10, 11, 15]. Solomon reported that outdoor spore populations could easily be evidenced indoors after a short period of time, which might obscure evidence of internal fungal sources [12]. This conclusion was based on the fact that indoor spore populations were measurable during the winter months when outdoor spore populations were nearly absent. Hirsch and Sosman conducted a one-year survey of mold in 12 homes in which they confirmed Solomon’s findings and concluded that homes are capable of supporting spore survival in the absence of outdoor spore contributions [6]. The conditions in various rooms of a home can range from high humidity and dampness in the basement to carpeting in the main living areas. These observations are consistent with and substantiate the fact that homes may be a source of perennial exposure to molds. Not all of these molds may be of clinical significance and their prevalence will vary with conditions and association with such things as carpeting, central air conditioning and pets in the home. These factors may, in some cases, be important sources of allergens for allergic subjects.

Hypersensitivity resulting from exposure to fungi is well documented [1]. It afflicts individuals in approximately 15 million households in the US who are under the preventative care of a physician. Hypersensitivity disorders range from acute reactions with mild discomfort to chronic debility requiring continual medication. An allergic response may also result
from exposure to the peptidoglycans with bacterial cell walls.

Antihistamines and other medications provide temporary relief from allergic discomforts but prophylaxis, if possible, would provide a safer, more efficacious alternative. Causal prophylaxis requires the elimination of microbial reservoirs in the home. Surfaces, such as carpeting and other textiles, are susceptible to microbial infestation. Most often it is prescribed that they be removed, encased in plastic and frequently cleaned, decreasing comfort and the aesthetic appearance of the surroundings. Envirizons, including HEPA filters, electrostatic precipitators and dehumidifiers are also recommended for prophylactic purposes. Early studies indicate that application of an immobilized antimicrobial agent, 3-trimethoxysilylpropyldimethyloctadecyl ammonium chloride (silane quaternary amine), should help eliminate the cost, inconvenience, loss of lifestyle and additional burdens imposed by these traditional remedies [8, 19, 20]. The antimicrobial agent transforms surfaces into active control devices that continuously inhibit microorganisms [7, 13, 17]. The antimicrobial durably bonds to textiles and other surfaces, rendering them micro biostatic [5, 9, 18]. Resident microorganisms are destroyed during application and reinfestation is controlled by residual activity.

This study was designed initially to investigate quantitative variances of airborne microorganisms in homes before and after treatment of carpeting with the immobilized silane quaternary amine. Concurrently, the symptoms and responses of mold-sensitive occupants in the homes were monitored.

**MATERIALS AND METHODS**

**Homes and Occupants**

A total of 19 homes in the metropolitan area of Cincinnati, OH, were selected for the study, at least 10 of which housed adolescent mold allergy sufferers. The homes were selected in conformance with the following criteria: (1) at least one family member had to be under the care of an allergist for at least one year and diagnosed as mold sensitive, (2) the attending allergist was asked to document clinical observations for at least 6 months, and (3) carpet and air conditioning were required in the main living areas of the home.

![Figure 1. Comparison of total Aeromicrobial retrievals and percent changes before and after antimicrobial treatment in residential homes.](image1.png)

![Figure 2. Comparison of total airborne retrievals of filamentous fungi and percent changes before and after antimicrobial treatment in residential homes.](image2.png)
Participants were asked to maintain daily symptom diaries to report changes, if any, in the degree and presence of allergic symptoms. They were trained to use peak flow meters and recorded expiratory peak flow breathing rates for morning and evening. Each allergy sufferer quantitatively noted his or her daily usage of oral, shot and inhalant medications during the entire study period.

**Antimicrobial Treatment of Carpeting**

The entire carpeted area of 18 homes selected for the study was treated with the silane quaternary amine antimicrobial agent per the manufacturer’s specifications by an authorized applicator [4]. One home (#19) was not treated and served as a control.

**Microbiological Sampling**

Sabouraud Dextrose Agar (SDA;BBL), in standard plastic petri dishes, was used for fungal retrievals by the open-plate gravity settling method of collection. Tryptic-Soy Agar (TSA;BBL), in standard plastic petri dishes, was used for bacterial retrievals by the same method. These media were chosen as general retrieval media for bacteria and fungi so comparative analysis of filamentous fungi and total microbiological load could be made respective to this sampling procedure. Ten plate pairs (SCA and TSA) were randomly placed at floor level throughout each home. The plates were exposed for 2 h, sealed and returned to the lab for incubation and enumeration. Microbiological sampling was completed immediately before antimicrobial treatment of the carpet and at one-week and one-month intervals after treatment. The retrieval plate locations in each home remained the same for each of the three sampling intervals. All samplings were done at the same time of day and every effort was made to duplicate air movement, human presence and activity.

**Culturing and Identification**

Plates were incubated at 30 C (SDA) and 35 C (TSA) for 72 h and 48h, respectively. Final counts were reported as those observed at the 72 h and 48 h intervals, with confirmatory readings after 7 days. Care was exercised to avoid secondary seeding of the plates by primary isolates of dry-spored types.

Colony counts were performed on all retrieval plates and colonies were differentiated into two morphological groups, filamentous fungi and all others. Identification of the isolates was not performed.

**RESULTS**

Aeromicrobiological retrievals were completed on the 19 homes initially selected for this study. Allergic symptoms and responses were monitored for 28 mold-sensitive occupants of the study homes.

Average total microbial retrievals in the homes prior to antimicrobial treatment of the carpet ranged fro 6 CFU’s per plate to 42 CFU’s per plate (Figure 1). After antimicrobial treatment, the average total microbial retrievals ranged from 1 CFU per plate to 20 CFU’s per plate.

The average total filamentous fungi retrieved prior to antimicrobial treatment ranged from 3 CFU’s per plate to 78 CFU’s per plate and 1 CFU per plate to 34 CFU’s per plate after treatment of the carpet (Figure 2). Thirteen of the 19 homes (68%) showed greater than 50% reduction in total aero microbiological populations following antimicrobial treatment of the carpeting. Ten of the 19 homes (53%) exhibited greater than 50% reduction in airborne fungus populations following treatment.

Analysis of the symptomatic responses from the mold-sensitive occupants in the homes revealed that 19 of 24 (79%) people recorded intermediate to significant improvement in their conditions. The improvements noted were fewer headaches, decreased congestion, better balance, decreased sinus problems, required medicine reduced or stopped and an overall better feeling. The remaining five allergy sufferers recorded essentially no changes in their allergic symptoms. Three of the original study participants reported being ill with colds or other infections during the evaluation period, and the allergy-sufferer in the
control house (#19) reported change of condition. These four original participants are not included in the calculation above.

**DISCUSSION**

The importance of microorganisms as indoor air pollutants in homes is now clear and their role in human health and well being is becoming better understood. It is well known that bioaerosols discharged by people and animals are primary contagious for respiratory diseases and airborne fungi play a key role in the health of mold-sensitive individuals. The presence and density of airborne microorganisms within a home is largely dependent on: (1) microbial incursion into the home, (2) proper nutritive and environmental conditions to permit survival and colonization of surfaces and (3) aerodynamic influences sufficient to aerosolize microbes, their spores or metabolic products. In order to control the microbial proliferation in a home, prophylactic techniques must be employed.

The limitations of open-plate gravity sampling of aeromicrobiological populations are recognized. By utilizing a sufficiently large number of sampling sites in each home, it is possible to make comparative interpretations and quantify variations of average plate retrievals at each site. Thus, it becomes possible to determine substantive changes in the microbiological quality of the air associated with a prophylactic technique.

In this study, we employed the use of an immobilized antimicrobial agent, a silane quaternary amine, as an intervention tool. This causal prophylactic strategy served to: (1) destroy microorganisms on a colonized surface, (2) activate the surface with a durable, bound antimicrobial to control reinfection, and (3) use the activated antimicrobial surface to reduce airborne microbial populations. The silane quaternary amine antimicrobial agent used in this study was previously used successfully to mediate a severe fungal problem in a school in Campbell County, KY, where all other chemical (cleaning and organo-quaternary amine antimicrobials) and physical (rebuilding and readjusting HVAC system and repair of sewer system) prophylactic techniques repeatedly failed to alleviate the problems of odor and health. Control of the microorganisms in the school environment subsequently resulted in a reduction of health complaints and problems [2].

Data from this residential study substantiates the use of the silane quaternary amine antimicrobial treatment to transform surfaces, such as carpeting, into active microbe control devices. At least two-thirds of homes showed 50-89% reduction in total aeromicrobiological populations and over half of the homes exhibited 50-98% reduction in airborne populations of fungi.

The overall results from monitoring 28 mold-sensitive individuals in the study homes suggested that the reduction of aeroallergens, such as bacteria and molds, accompanied a significant decrease in symptomatic allergic responses. However, complete linkage of the positive human responses with increased aeromicrobial reductions is difficult because other microbial reservoirs besides carpeting may have been present in the homes and were not affected by the treatment. Expiratory peak flora rate, an important measure of response in an allergic individual, showed no correlation to variation of filamentous fungi or total aeromicrobiological load, with the testimonial statements of the subjects, or with drug usage patterns. This indicates the need for a more sensitive quantitative human response measurement technique. The nature of human response is a threshold event and as such is individualistic.

Use of the data from this preliminary study may show some obvious trends, but extension of the data to formulate broad conclusions is risky due to the quantitative sensitivity of the aeromicrobiological sampling technique. The burden of these initial anecdotal results, the possibility of psychological interferences, and the need for more controlled data gathering in the home demands that a more detailed double-blind study be undertaken to evaluate the utilization of this unique technology to mediate indoor aeromicrobiological contamination.
REFERENCES


The detection of microbial cells is an active field of research, and several monitoring techniques based on electrical conductivity or electrical capacity (16), calorimetry (17), and friction and pressure drop (18), as well as sound (19) and electromagnetic radiation, have been developed. We recently explored microbial settlement on a 2-D photonic crystal fabricated in silicon on a transparent and flexible substrate. A particularly promising and surface-sensitive variant of the technique is attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy, where the penetration depth of the evanescent field is ≤1 μm. Environmental Monitoring of Pharmaceutical Manufacturing Facilities. 3. Microbial Monitoring is a program designed to demonstrate the control of viable (living microorganisms) and non-viable particles in critical areas. These areas include clean-rooms for drug fill/finish, formulation tank rooms, laminar flow hoods, biological safety hoods, isolators, Intravenous (IV) compounding areas and sterile packaging. 4. Viable monitoring - Testing for the detection and enumeration of bacteria, yeast and mold. It includes the monitoring of personnel, air and areas for contamination. Non-viable monitoring - A reference for particle counts measured by a laser counter. Environmental monitoring is critical to the protection of human health and the environment. As the human population continues to increase, as industrial development and energy use continues to expand, and despite advances in pollution control, the continued production of pollution remains inevitable. Thus the need for environmental monitoring is still as great as ever. Continued advances in the development, application, and automation of monitoring devices are needed to enhance the accuracy and cost-effectiveness of monitoring programs. Equally as important is the need to produce more scientists.